VA Summer Epidemiology Session
Developing Scientific Research Proposals (Grant Writing)

Session 3 (Readings)

Background and Significance; Preliminary studies

A. Background / Literature Review Overview

B. Background / Literature Review Examples

C. Significance Overview

D. Significance Examples

E. Preliminary Studies

F. Preliminary Studies Examples

The **Background** section is, simply stated, a comprehensive but targeted literature review. In this section, you demonstrate your familiarity with the published and, whenever possible, ongoing research relevant to you specific aims. (Remember your Specific Aims?) The **Significance** section outlines the importance or relevance of the work you proposed. The **Preliminary Studies** section demonstrates your ability to complete the proposed work.

A. Background / Literature Review

Beware an unfocused, rambling, unbalanced literature review. Understand the literature and organize it in some meaningful way (try topic sentences!). Summarize the major results, give more detail on studies close to your specific aims, and give as much detail as you can on the strengths and weaknesses of studies that motivate your hypotheses.

Try several techniques. First, in a short, introductory paragraph state what areas you will cover and why. You cannot cover everything relevant to your proposal, so pick and choose carefully

and justify to reviewers why you have selected the topics that follow. This makes it easier for the reader to follow your organization and arguments. It also shows that you have a broad understanding of the science, but are focusing on only those areas that are important to your proposal. Second, make liberal use of heading, subheading, and sub-subheadings. This too helps the reviewer know the topic being covered, and it saves valuable space compared to using text for transitions between content areas. Third, if the literature is vast, use tables to summarize it. A secondary benefit for this type of review is that you can usually turn this work into a published literature review with only modest additional effort. *Fourth, make it **painfully** clear how your Background and Significance section motivates your specific aims.* Do not be shy. Refer back to your specific aims as many times as necessary. And remember, the reviewer will likely not know the subject area as well as you do. Clearly articulated statements leading to a justification of your proposal are very helpful.

Below are excerpts from Background sections. These illustrate the points above.

B. Background / Literature Review Examples

Example 1: Cohort Study of Dietary Supplements and Cancer Risk

Overview and rationale for study. There is extensive evidence for a role of plant foods in lowering risk of human cancers (1-4). The recent edition of Cancer Causes and Control devoted to a review of nutrition and cancer concluded that the current evidence demonstrates a protective effect of vegetable consumption, and less definitively fruit consumption, on almost all major cancers (4). The editors concluded that the evidence was convincing or suggestive for cancer of the lung, breast, prostate, large bowel, oral cavity, esophagus, stomach, liver, pancreas, larynx, endometrium, cervix, ovary, bladder and kidney. Although they made no attempt to summarize the evidence with respect to micronutrients, epidemiologic, animal and in vitro studies provide evidence that there are a very large number of bioactive compounds in food that may influence the likelihood of cancer. Included among potential agents are a variety of vitamins (e.g., retinol, vitamin C, vitamin E, folic acid), their precursors (e.g., β-carotene), and minerals (e.g., calcium, selenium).

High prevalence of supplement use. Consumers have responded to the extensive media coverage of micronutrient and disease relationships. About half of those in the 1992 National Health Interview Survey had taken some type of supplement, and daily use among men and women over age 45 was 22% for multivitamins, 11% for vitamin C, 7% for vitamin E, and 9% for calcium (13). Use is significantly higher in the western U.S. than other regions (14, 15). Retail sales increased by 19% between 1987 and 1992 to approximately $3.7 billion (16).

Exposures of interest: Sources and biologic mechanisms

Vitamin C. The greatest dietary sources of vitamin C are fruits and vegetables; average intake from food is about 100 mg for men over age 50 and 90 mg for women over age 50 (17). Individual supplements of vitamin C provide 5-10 times the amount found in diet (250-1000 mg) while a multivitamin provides the US RDA (60 mg).

Reviews of studies of vitamin C suggest several mechanisms by which vitamin C may reduce cancer risk (18-22). Ascorbate could reduce cancer by its action as an antioxidant, by protecting cells against oxidative DNA damage due to normal metabolic processes. Vitamin C may prevent cancer, especially in the bladder or stomach, by reducing the formation of nitrosamines (18, 19, 23). Furthermore, ascorbate may enhance immune response and enhance connective tissue integrity. In in vitro studies, vitamin C has been shown to cause regression of tobacco-induced malignant changes in hamster lung cells. Experiments involving the feeding of vitamin C to animals to which carcinogens have been administered have mainly shown a protective effect or no evidence of effect.

Vitamin E. The major sources of vitamin E (which is fat-soluble) are vegetable oils and margarine. Dietary intake among men over age 50 is approximately 27 IU and 17 IU for women over 50 (17). Individual supplements of vitamin E are typically 20 times this amount (400 IU dl α-tocopheryl acetate), while a multivitamin contains the US RDA of 30 IU. Reviews of studies suggest several functions of vitamin E may be anti-carcinogenic (18, 21, 22, 24-26). Vitamin E is an intracellular antioxidant, and protects polyunsaturated fatty acids in cell membranes from oxidative damage. *Vitamin E at 200 mg/day improves immune response in elderly subjects (26a).* Another possible mechanism for vitamin E relates to its capacity to enhance the anti-oxidant role of selenium. Vitamin E also inhibits the formation of nitrosamines (19, 23), especially at low pH. In vitro research has suggested a role in reducing the expression of c-myc and H-ras (26). Animal experiments on the effect of dietary vitamin E in cancer prevention are, overall, inconclusive.

Calcium. Calcium intake from diet, primarily from dairy products, is about 725 mg for men over age 50 and 575 mg for older women (17). The most common dose for individual calcium supplements is 500 mg.

Calcium has been linked primarily to colon cancer. Calcium consistently reduces colon cancer in experiments in animals (27-33), most likely by normalizing colonic crypt cell proliferation kinetics (34-37). Several small clinical trials (38-42) suggest similar effects in humans; however, other trials do not (43, 44). The first full-scale trial of calcium on proliferation in humans showed no effect on overall proliferation, but a significant downward shift of the proliferative zone of the epithelial crypt (45). Possible mechanisms include the binding of bile acids (which are thought to be promoters of colonic neoplastic change) to form inert soaps (34) and the direct induction by calcium of terminal differentiation of the colonic epithelial cells (43, 46-48).

Multivitamins. Multivitamin pills include about 10 vitamins, and most consumers take multivitamins with minerals which also include about 10 minerals. Table *1* gives a common formulation. Multivitamins generally contain 100% of the RDA for those micronutrients for which there are recommendations, except for calcium and certain other minerals, which are too bulky to include more than about 10-20% of the RDA. On average, men and women consume about the RDA of most nutrients from food (17), therefore taking multivitamins approximately doubles the intake of these nutrients (except for calcium). In addition to the effects of vitamin C and E discussed above, other micronutrients in multivitamin/mineral pills have functions that may reduce the risk of cancer.

Retinol (preformed vitamin A) has always been a constituent of multivitamin preparations. Because of the interest in the possible anti-cancer role of β-carotene (a retinol precursor), β-carotene has now been added to multivitamin pills. There is extensive research on the relationships between carotenoids and retinol and cancer (18, 19, 49-53). The functions of vitamin A include a role in the regulation of epithelial cell differentiation; because lack of differentiation is a feature of cancer cells, an etiologic role for inadequate vitamin A is plausible (54, 55). In vitro, retinoids reverse pre-malignant changes or prevent malignant changes in cells exposed to a variety of carcinogens. Additionally, β-carotene is an antioxidant. However, the recent large β-carotene trials found increased lung cancer risk among high risk individuals, possibly due to adverse effects among those with many initiated cells (56). Both retinoids and carotenoids may be important in cell-cell communication—increasingly thought to be crucial in the loss of tissue architecture universal in the cancer process (57). The majority of animal research on vitamin A and cancer has involved retinoids and not carotenoids. Vitamin A deficiency in animals leads to pre-malignant changes and enhances the development of chemically-induced cancers; in a few animal studies, retinoids enhanced tumor production (18, 19, 49, 53).

Table 1. Formulation of One-A-Day® Vitamins and Minerals

Vitamins	Quantity	Minerals	Quantity
Vitamin A[a]	5000 I.U.[b]	Iron (Elemental)	18 mg.[b]
Vitamin C	60 mg.[b]	Calcium	130 mg.[c]
Thiamine (B_1)	1.5 mg.[b]	Phosphorus	100 mg.[d]
Riboflavin (B_2)	1.7 mg.[b]	Iodine	150 mcg.[b]
Niacin	20 mg.[b]	Magnesium	100 mg.[e]
Vitamin D	400 I.U.[b]	Copper	2 mg.[b]
Vitamin E	30 I.U.[b]	Zinc	15 mg.[b]
Vitamin B_6	2 mg.[b]	Chromium	10 mcg.[f]
Folic Acid	0.4 mg.[b]	Selenium	10 mcg.[f]
Vitamin B_{12}	6 mcg.[b]	Molybdenum	10 mcg.[f]
Biotin	30 mcg.[b]	Manganese	2.5 mg.[f]
Pantothenic Acid	10 mg.[b]	Potassium	37.5 mg.[f]
		Chloride	34 mg.[f]

[a]As Acetate and β Carotene
[b]100% RDA
[c]13% RDA
[d]10% RDA
[e]25% RDA
[f]No RDA established

MacGregor et al have discussed the possible role of folic acid as an anti-carcinogenic agent (58). In vitro and animal studies have shown that folic acid deficiency causes increased micronuclei formation and increased chromosomal damage. The expression of certain chromosomal fragile sites, which are associated with oncogenes and breakpoints thought to be relevant to specific cancers, has also been shown to be increased in folic acid deficiency. Further, dysplasia exhibited by cervical and lung cells may be reversed by administration of folic acid. A specific role for folate has been proposed in reducing the likelihood of hypomethylation of DNA, an established step in the progression of colon carcinogenesis and of possible wider significance (59, 60).

Studies suggest several anti-carcinogenic effects of selenium (18-20, 22, 24, 25). The most clearly defined function of selenium is its role as a cofactor for glutathione peroxidase, an enzyme that protects against oxidative tissue damage. It also suppresses cell proliferation at high levels. Selenoglutathione has been shown to reduce tumor growth. Selenium may enhance immune response or alter the metabolism of carcinogens towards production of less toxic compounds. Selenium decreases the mutagenicity of many carcinogenic compounds, as determined by the Ames test. On the other hand, several selenium compounds, specifically selenites, damage DNA. The majority of animal studies involving the feeding of very high doses of selenium have found an inhibition of induced carcinogenesis.

Randomized trials of supplements. *We recently (1997) published a review in Cancer Causes and Control of the randomized trials and observational studies of the associations of vitamin supplements to the risk of all types of cancer (4a). Below we summarize findings from chemoprevention trials (Table 2) and observational studies (Tables 3 and 4) on vitamin supplements and total cancer mortality and incidence; and lung, prostate, breast, and colon cancers. (Observational studies of total intake of nutrients (diet plus supplements) are not presented.)*

In response to the evidence for a role of micronutrients in cancer prevention, the National Cancer Institute has developed a large chemoprevention program. The most tested agent was β-carotene. Those studies have shown not only that β-carotene as a supplement in pharmacologic doses does not reduce risk of total mortality or death from cancer (7-10), but both the ATBC study in Finland (7) and the CARET study (10) found that it actually may increase risk of lung cancer in high-risk populations. *However, in the ATBC trial, prostate cancer incidence was significantly lower in the α-tocopherol (vitamin E) group than in the group not receiving α-tocopherol (7).* In the Chinese general population study of high-dose multivitamins, there was modest reduced risk of mortality and cancer death among those randomized to β-carotene, selenium and α-tocopherol (5). *In a small randomized clinical trial of 1312 patients with a history of basal cell or squamous cell carcinomas of the skin, there was no protective effect of selenium supplementation on risk of skin cancer (11). However, analyses of secondary end-points found statistically significantly reduced risks of cancer mortality, overall cancer incidence, and the incidence of lung, prostate, and colorectal cancers.*

Table 2. Randomized Clinical Trials of Vitamin/Mineral Supplements and Total Mortality and Risk of Cancer of the Lung, Prostate, Breast and Colon

Author	Sample	Follow-up	Supplement	Relative Risk for Supplementation[b]
Blot 1993 (5)	29,584 adults in China	5.25 years Total Mortality (2127 deaths) Cancer Deaths (792 deaths)	5000 IU Retinol + 22.5 mg Zinc 3.2 mg Riboflavin + 40 mg Niacin 120 mg Vitamin C + 30 mcg Molybdenum 15 mg β-carotene + 50 mcg Selenium + 30 mg α-tocopherol	1.0 (0.9-1.1), mortality 1.0 (0.9-1.1), cancer 1.0 (0.9-1.1), mortality 1.0 (0.9-1.1), cancer 1.0 (0.9-1.1), mortality 1.1 (0.9-1.2), cancer 0.9 (0.8-1.0), mortality 0.9 (0.7-1.0), cancer
Li 1993 (6)	3,319 adults with esophageal dysplasia in China	6 years Total Mortality (234 deaths) Cancer Deaths (176 deaths)	High dose Multi-vitamin with Minerals	0.9 (0.7-1.2)[a] 1.0 (0.7-1.3)[a]
The Alpha-Tocopherol, Beta-Carotene Study (ATBC) 1994 (7)	29,133 male smokers in Finland	5-8 years Total Mortality (3590 deaths) Lung Cancer (876 cases) Prostate Cancer (250 deaths) Colon Cancer (149 deaths)	20 mg β-carotene 50 mg α-tocopherol	1.1 (1.0-1.2), mortality 1.2 (1.0-1.4), lung 1.2 (ns), prostate 1.0 (ns), colon 1.0 (0.9-1.1), mortality 1.0 (ns), lung 0.7 *(0.001), prostate*[d]

				0.8 (ns), colon
Greenberg 1996 (8)	1,188 men and 532 women in US	8.2 years Total Mortality (285 deaths) Cancer Deaths (82 deaths)	50 mg β-carotene	1.0 (0.8-1.3)[a] 0.8 (0.5-1.3)[a]
Hennekens 1996 (9)	22,011 male physicians in US	12 years Cancer Incidence (2566 cases) Cancer Deaths (766 deaths)	50 mg β-carotene on alternate days	1.0 (0.9-1.1) 1.0 (0.9-1.2)
Omenn (CARET) 1996 (10)	18,314 smokers, former smokers, and asbestos workers in the US	4 years Total Mortality (764 deaths) Lung Cancer (388 cases) Prostate Cancer (300 cases)	30 mg β-carotene + 25,000 IU Vitamin A	1.2 (1.0-1.3), mortality 1.3 (1.0-1.6), lung ≈1.0, prostate
Clark 1996 (11)	*1312 patients with a history of basal cell or squamous cell carcinomas of the skin*	*6.4 years* *Cancer mortality* *All sites (86 deaths)* *Cancer incidence* *Total carcinomas(163 cases)* *Lung cancer (48 cases)* *Prostate cancer (48 cases)* *Colorectal cancer(27 cases)*	*200 mcg selenium supplied as a 0.5 g high-selenium brewer's yeast tablet*	*0.5 (0.3-0.8), mortality* *0.6 (0.4-0.8), total* *0.5 (0.3-1.0), lung* *0.4 (0.2-0.7), prostate* *0.4 (0.2-0.9), colo-rectal*

[a]Adjusted relative risk, see original studies for details. [b]ns means non-significant [d]*P-value calculated from published data*

Example 2: <u>Diet and Genetic Risk for Lung and Prostate Cancer</u>

High-fat, low-fiber diets are associated with increased risk of several major cancers, most importantly cancer of the colon, rectum, breast, and prostate.[1] The scientific evidence supporting a causal relationship of diet with all of these cancers is not yet conclusive, but there is growing scientific consensus that public health nutrition interventions are warranted.[2] As part of its year 2000 goals, the National Cancer Institute has recommended that Americans reduce their intake of fat to less than 30% of total energy and increase their intake of fiber (from fruits, vegetables, and whole grains) to at least 20 grams per day.[3] For most persons, adopting diets consistent with recommended guidelines would require major and sustained lifestyle changes. The design of public health programs that can facilitate these dietary changes is a considerable scientific challenge.

The substantial research literature on promoting dietary change is based mostly on intensive, professionally administered interventions. One of the most successful diet change intervention studies, the Women's Health Trial (WHT), was developed and tested in collaboration with nutritional scientists at the Cancer Prevention Research Program (CPRP). The WHT diet intervention model is now the basis of many clinical trials, including the WHT Feasibility Study in Minority Populations and the Women's Health Initiative. In contrast, there is very little research on low-intensity interventions in healthy persons. The challenge for public health scientists is to translate the effective components of intensive interventions into programs that are practical for public health application.

Self-help dietary change programs, used either alone or as a component of comprehensive community-level programs, have important advantages for public health interventions. As will be reviewed in detail below, self-help interventions: 1) are preferred by many people over individual or group interventions; 2) can be as effective as more intensive interventions; and 3) when at least minimally effective are far more cost-effective than individual interventions. A synthesis of results from studies of consumer nutrition behavior, clinical nutrition interventions, smoking self-help interventions, and a very small number of clinical trials of self-help nutrition materials in healthy persons, suggests ways in which self-help diet change programs can be delivered to large proportions of the population, at low cost, and with meaningful effects. We therefore propose to build upon this work to develop and evaluate a comprehensive, self-help diet-change intervention. To justify the design and hypotheses of this proposed research, the review that follows describes: 1) research on the use of dietary change self-help materials; 2) research on the design and efficacy of self-help materials; 3) population-level effects from small dietary change; and 4) previous studies by this research team leading to this proposal.

Use of Dietary Change Self-Help Materials

Many people prefer self-help materials as an approach to health promotion behavior change interventions,[4] and this appears especially true for diet. For example, 36% of adults in both control and intervention communities in the Stanford Five-City Project used diet change self-help materials.[5] In the Minnesota CANDI project,[6] over 18% of an entire community enrolled in a mailed, self-help diet intervention program. In both these studies, use of self-help materials was more frequent among women, persons over 35 years old, homemakers, and persons better educated. Use of self-help materials is also related to their mode of distribution. For example, Kishchuck et al.[7] found that use (assessed as recognition and learning) of self-help materials is much greater when materials are sent to individuals directly rather than simply made available upon request. Overall, studies suggest that self-help materials can be very popular, but that direct distribution and special efforts to target young adults, men, and persons less well educated will be important to reach diverse segments of a community.

Efficacy of Self-Help Materials

Self-help diet change interventions have been shown to be as effective as more intensive, interpersonal interventions for control of diabetes[8] and hypercholesterolemia,[9] weight loss,[10] and in promoting very low-fat diets.[11] In addition, several studies have shown that the addition of even minimal non-informational components, including two important components of this proposal, personal contact[12] and dietary assessment with behavioral feedback,[4] can enhance the effectiveness of diet self-help materials. These findings are consistent with the far larger literature on self-help smoking cessation programs.[13] For example, Curry et al.[14] demonstrated that adding personalized, specific….. These results, in particular the importance of addressing motivation, specific behavioral targets and personalized behavioral feedback, need to be applied in studies of nutrition self-help interventions.

Three published and one unpublished randomized studies have evaluated whether self-help materials could reduce fat and/or increase fiber intake in healthy persons. Baron et al.[17] randomized 368 persons in a general medical practice to receive either a diet change booklet

with nurse counseling or no intervention. They found significant and sustained changes in some diet behavior, especially substitution…

A recent report of a study in North Carolina addressed whether personalization of self-help nutrition information increases intervention efficacy[61]*. This study recruited clinic patients, and after successful baseline interviews randomized them into control, standard or personalized intervention groups. The interventions were one-time mailings, and personalization consisted of computer-generated messages based on participants' stage of dietary change. Follow-up was at 4-months post randomization, based on a 28-item food frequency that generates a score for fat and servings of fruits and vegetables. There were significant and incremental decreases in fat comparing the standard and personalized interventions to controls, but no change in fruit and vegetable intakes. Results of this study support the main hypothesis of this study, that personalization can significantly enhance intervention efficacy. There are two important shortcomings in the North Carolina study that are address in this proposal: (1) In the North Carolina study, personalization was based on relatively little information about participants dietary habits. Intervention was based on stage of dietary change, but there was no strong underlying model of how persons adopt and maintain new dietary behavior (see Section 4.d.1.); and (2) The single evaluation method, a short food frequency questionnaire, is subject to significant bias (see Section 3.d. and Table 3), and a 4-month follow-up cannot address questions related to long-term effectiveness of personalized intervention approaches or processes involved in dietary change. Despite these shortcomings, the North Carolina study provides strong support to the generalizability of results from the smoking literature on the importance of enhancements to self-help behavior change interventions.*

Population-Level Effects of Small Dietary Change

There is disagreement in the scientific community about the importance and interpretation of small changes in dietary habits. The crux of this argument lies in the apparent inconsistency between intervention effects that are meaningful ***clinically*** *versus those that have important* ***public health*** *impact*[20]*. Clearly, a difference of one or two percentage…*

Example 3: Alternative Therapies for Back Pain

Public Health Importance of Back Pain

Back pain is one of the most important health problems in the United States and other developed countries. It has been estimated that more than 50% of adults are bothered by back pain each year (Sternbach, 1986) and 70% to 80% of adults are afflicted by it at some time in their lives (Frymoyer, 1988). Back symptoms are the leading cause of visits to orthopaedic surgeons and the second leading reason for visit to all physicians (Cypress, 1983; Hart, 1995). Until recently, "medical back problems" was the second most common medical diagnosis-related group (DRG) for all hospital discharges. Among surgical DRG's, back and neck procedures ranked only behind cesarean sections and tubal ligation (Graves, 1987; 1987 National Hospital Discharge Survey, NCH unpublished data). Back pain is the most costly ailment of working-age adults, with 1985 earnings and productivity losses exceeding $5 billion for men alone (Salkever, 1985). It has been estimated that total annual costs of back pain (direct medical costs plus lost productivity

and compensation) in the United States are between $50 and $100 billion per year (Frymoyer, 1991).

Although it is a common problem, many physicians feel poorly prepared to manage low back pain when they enter practice and often feel frustrated by patients with back pain (Cherkin, 1988). This frustration derives in part from diagnostic ambiguity and the paucity of treatments that have been shown to be more effective than reassurance and natural history alone (Nachemson, 1979; Deyo, 1983a). Low back pain patients are relatively dissatisfied with medical care, especially in comparison to care received from non-medical health care professionals (Cherkin, 1989; Greenfield, 1975; Kane, 1974; Overman, 1988; Carey, 1995).

Diagnostic Ambiguity

There are many potential causes of low back pain, but in most cases, a precise pathoanatomic diagnosis is unattainable. This is because of the weak associations among symptoms, pathoanatomic changes and imaging results. Serious causes such as infections and malignancy are unusual, and it has been estimated that up to 85% of cases are caused by degenerative changes in the discs and facets or musculo-ligamentous injuries (often labeled "lumbar" strain) (White, 1982). Radiographic procedures may be misleading because many "abnormalities" are as frequent among persons without back problems as in patients with back pain (e.g., many degenerative changes, spondylosis, congenital anomalies, and facet joint changes) (Deyo, 1986a). Computed tomography, myelography and magnetic resonance imaging reveal pathology such as herniated disks and spinal stenosis in 20%-30% of normal persons with no history of back pain or sciatica (Hitselberger, 1968; Wiesel, 1984).

There is even controversy about the existence of (or criteria for) many commonly used diagnoses. For example, the finding of muscle "spasm" is not reproducible even among experts in the same clinic (Waddell, 1982). The existence of trigger point syndromes prompted such debate that opposing chapters had to be written for an Institute of Medicine report on chronic pain (Osterweis, 1987). The existence of "fibrositis" is challenged by some (Hadler, 1984), and there is little agreement on the definitions of "disk disruption syndrome", "segmental instability", or "sacroiliac strain."

Even though a definitive diagnosis may be unattainable, a clinically meaningful categorization of patients is possible. The Quebec Task Force on Spinal Disorders (Spitzer, 1987) recommended categorizing patients on the basis of pain duration, radicular symptoms, work status and (if available) imaging results. In our study, patient questionnaires will permit general classification of each patient according to the Quebec system, although imaging results (which, as noted above, can be misleading) will not be available.

Importance of Functional Outcomes

The choice of outcome measures in clinical trials of back pain therapy deserves careful attention. Many physiologic or laboratory measures are attractive because of their apparent "hardness" (e.g., range-of-motion measures, spinal fluid endorphin levels, or paraspinal muscle EMG activity). However, these measures have little inherent value to patients, and it has been repeatedly demonstrated that they are only weakly related to pain symptoms or daily functioning (Nouwen, 1983; Ward, 1986; Fordyce, 1984; Lankhorst, 1985). Since we are primarily concerned with pain and functional ability, we must measure these directly, rather than with

weak proxies. Fortunately, substantial progress has been made in the past 15 years in the measurement of daily functioning for ambulatory patients with low back pain. Instruments such as the Sickness Impact Profile and its shorter back-specific adaptation (the "Roland" scale) have demonstrated reasonable validity, reliability, and responsiveness to clinical changes (Deyo, 1983b; Roland, 1983a and 1983b; Deyo, 1986). We propose to use the brief Roland scale in our clinical trial.

Efficacy of Treatments for Back Pain

Due to the lack of consensus about optimal treatment, there are large geographic and inter-provider variations in how back pain is diagnosed and treated (Wennberg, 1984 and 1987; Cherkin, 1988, 1994a, 1994b, 1995; Battie, 1994; Taylor, 1994; Carey, 1995). Many non-surgical treatments are used to treat back pain, but few have been shown to be effective in randomized trials. Spinal manipulation, certain medications and possibly exercise, are the only conservative treatments for back pain that have been found effective in multiple randomized trials, although many treatments (including acupuncture and massage) have never been well studied (Deyo, 1983a; Bigos, 1994). No randomized trial designed to evaluate the efficacy of massage could be identified in the peer-reviewed literature, although massage has been used as a comparison treatment in a few studies.

Efficacy of Acupuncture

Acupuncture is defined here to include a variety of procedures involving the insertion of needles without medication ("dry needling") into cutaneous and subcutaneous tissues, muscles or ligaments. The Chinese have used acupuncture for the treatment of various problems, including back pain, for more than 2,000 years. Traditional Chinese acupuncture is based on Chinese philosophy which specifies where needles should be placed to treat persons found to have specific diagnoses. Traditionally, the inserted needles would be rotated to produce a noxious stimulus although electrical stimulation is now often used. Neither the Chinese diagnoses nor the locations of the Chinese meridians where needles are to be inserted correspond to Western medicine's system of diagnosis or understanding of the location of neural pathways. Other forms of dry needling ignore the Chinese meridians and involve insertion into tender spots or other areas. A primary therapeutic objective of acupuncture is to reduce pain although it is also used for other problems such drug addiction and as an anti-emetic.

A number of putative mechanisms have been proposed to explain how acupuncture relieves pain (Pomeranz, 1989). It is possible that acupuncture operates according to the gate theory of pain proposed by Melzack and Wall (1965). This theory hypothesizes that one type of sensory input could be inhibited in the central nervous system by another kind of sensory input. It is possible that acupuncture stimulates production of endorphins and serotonin and acetylcholine within the central nervous system, enhancing analgesia (Pomeranz, 1976). Another theory "implies that noxious stimulation of heterotopic body areas modulates the pain sensation 'originating' in areas where a subject feels pain." (Willer, 1984; LeBars, 1988). This theory could explain why the location of stimulation is unimportant as long as the stimulation is noxious (Le Bars, 1988).

Despite the fact that acupuncture has been used to treat pain for more than two millennia, its effectiveness remains unclear. A 1990 meta-analysis of acupuncture for chronic pain concluded, "no studies of high quality seem to exist," and therefore, "no definitive conclusions on the

efficacy of acupuncture in the treatment of chronic pain can be drawn." (ter Riet, 1990). This review identified ten trials of acupuncture specifically for low back pain, including several studies which provided almost no information about the study methods. The sample sizes in these studies was typically very low with only two studies reporting more than 50 subjects per group. Four studies reported negative results and six positive results. The greatest deficiencies of published trials of acupuncture for chronic pain were: inadequate sample sizes, high percentages of subjects lost to follow-up, inadequate follow-up periods and use of unsophisticated techniques for measuring outcomes (ter Riet, 1990).
The guidelines for low back pain recently published by the U.S. Agency for Health Care Policy and Research identified 6 randomized trials evaluating the use of acupuncture for chronic low back pain and none for acute low back pain. This review concluded that although "outcomes were better for the acupuncture group than for nontreatment control groups," it did not seem to matter where the needles were placed (Bigos, 1994). All the studies were found to have had methodologic flaws.

No previous study of acupuncture for chronic low back pain has attempted to determine how the introduction of acupuncture services into a managed care organization would affect patient outcomes or costs. Unlike chiropractic, acupuncture is rarely covered by insurance and for this and other reasons, has not been very accessible to persons with chronic low back pain. It is becoming increasingly evident that Americans' use of alternative treatments, especially for chronic back problems, is extremely common (Eisenberg, 1993). Although acupuncture is used less frequently for back pain than chiropractic and massage, its use will increase if the trend toward expansion of "alternative" treatments continues. Many persons with chronic back pain make frequent use of various diagnostic tests and conventional treatments but often fail to realize much benefit. Patients, providers and health care managers would greatly benefit from knowing if acupuncture provides an effective (and cost-effective) alternative to conventional treatments for persons with chronic low back pain.

In summary, although a number of randomized trials have evaluated the efficacy of acupuncture for chronic low back pain, the quality of these studies has generally been poor. Pending confirmation by more scientifically rigorous studies, it appears that persons with chronic back pain may benefit from acupuncture, although it may not matter where the acupuncturist inserts the needles. Studies with larger sample sizes, more sophisticated outcome measures, longer follow-up periods and higher follow-up rates are needed before firm conclusions can be drawn. The proposed study is designed to overcome the problems plaguing most earlier studies by the inclusion of a large number of subjects, the use of state-of-the-art outcome measures, the measurement of long-term outcomes, and the use of methods that have succeeded in achieving high follow-up rates. In addition, the proposed study will address a question of great practical importance to patients, providers and insurers: Regardless of the underlying mechanism, does acupuncture, as it is commonly practiced, provide an effective (and cost-effective) alternative to the existing treatments available for persons with chronic low back pain?

Efficacy of Massage Therapy

Even though massage is the second most frequently used "alternative" treatment for back pain (chiropractic was first) (Eisenberg, 1993), no randomized trials designed to evaluate the efficacy of massage for low back pain could be identified. Various types of massage have been included

as one of two or more "control" treatments in randomized trials of bed rest for acute back pain (Postacchini, 1988), manipulation for acute back pain (Godfrey, 1984), manipulation for subacute back pain (Pope, 1994), manipulation for chronic back pain (Waagen, 1986 and Arkuszewski, 1986), and intensive exercise for chronic back pain (Hansen, 1993). Massage alone was used as the control treatment in a randomized trial of sacroiliac joint mobilization for persons with acute sacroiliac joint pain (Wreje, 1992). This small study (18 in mobilization group and 21 in massage group) found no difference between massage and mobilization in terms of effect on pain but the mobilized group had significantly less sick leave and use of analgesics.

Example 4. VA Biracial Cohort of Prostate Cancer

In 1999 there were approximately 200,000 new cases and 40,000 deaths from prostate cancer. Annual costs associated with the diagnosis and treatment of prostate cancer exceed $13 billion annually. Prevalence, costs and practice patterns in veterans are not known. However, if estimates from non-VA settings are utilized, up to one million veterans at a cost of $2 billion annually could be affected.

Ninety-five percent of prostate cancer is diagnosed in men between 45 and 89 years of age with a median age of diagnosis of 72 years. The age adjusted incidence and death rates from prostate cancer vary widely from country to country as well as between racial-ethnic groups. The risk of a man's developing prostate cancer depends primarily on his age, race, and number of affected relatives. The rates are highest for African-Americans, intermediate for U.S. Whites and lowest in Orientals.

The natural history of prostatic carcinoma is largely unknown and may vary according to race (Optenberg, 1995). Prostate cancer may be unique among solid tumors because it essentially exists in two forms: histologic or latent form (which can be identified in up to 70% of men over the age of 80) and the clinically evident form which affects approximately one out of six men in their lifetimes (Carter, 1990). Subclinical forms of prostatic carcinoma are present in a substantial proportion of the male population. Regional, national, and racial variations in the incidence of subclinical and invasive prostate cancer suggest the role of hereditary, dietary, lifestyle and environmental factors. Approximately 9% of prostate cancers and 45% of cases in men younger than 55 years of age can be attributed to a cancer susceptibility gene that is inherited as a rare autosomal dominant allele (Carter, 1992). The role of tumor suppressor genes and oncogenes continues to be defined. In particular mutations of the p53 gene and the androgen receptor may be important in the development of metastatic and hormone refractory disease (Navone, 1993).

The incidence and mortality for prostate cancer, however, are substantially higher for African-Americans compared to White men (Ross, 1987; Ries, 1994; Baquet, 1991). Reasons for these differences are unknown but possibly include genetic and lifestyle factors such as diet, smoking, and physical activity. The Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute reported summary incidence prostate cancer rates between 1988-1992 that were seven times higher among African-Americans (180.6 per 100,000) than among Koreans (24.2) (Miller et al, 1994). Age-adjusted rates per 100,000 population under age 65 were 48.3 for African-American men compared to 32.0 for White men. Age-adjusted rates per

100,000 population age 65 and over were 1458.9 for African-Americans men compared to 1120.3 for White men. African-American men also have the highest mortality rates in the world associated with prostate cancer(Kurihara, 1989). Age-adjusted mortality rates were more than twice and high in African-American men compared to White men for both younger and older age groups. There are no clear reasons why incidence of prostate cancer and mortality are higher in African-Americans. Possible explanations may include genetic factors, medical factors such as benign prostatic hyperplasia and vasectomy, lifestyle factors such as smoking, occupational exposure, socioeconomic factors, and dietary factors(Haas, 1997).

One of the primary objectives to establish a large multi-racial cohort is to explore differences in the risk factors for prostate cancer among Whites, African-Americans and Hispanics. Rates of prostate cancer in African-American men are up to 50% greater than in White men, and mortality due to prostate cancer is twice as high. The explanation for these racial differences may include genetic factors, medical factors such as benign prostatic hyperplasia and vasectomy, lifestyle factors such as smoking, occupational exposure, socioeconomic factors and, perhaps most importantly, dietary factors. Research conducted mainly in cohorts of white-collar Caucasian males has suggested that there may be important dietary and other lifestyle determinants of prostate cancer. Increased consumption of animal fat, low dietary lycopene intake (a vitamin A precursor), high calcium intake, or low fruit consumption are all factors postulated to result in an increased risk of prostate cancer. The differential rates of these factors by race may explain, in part, the racial differences in prostate cancer incidence and mortality.

Ongoing epidemiological studies have not been able to recruit sufficient numbers of African-American men to study the relationship between lifestyle and prostate cancer. This is largely due to the inability to identify racial groups prior to recruitment efforts, as well as African-Americans' longstanding distrust of the medical community. Because of this, our current understanding of risk factors for prostate cancer has been largely limited to studies of white-collar Caucasian men in professional health studies. Studies have not been designed to examine reasons for the differences in incidence of prostate cancer between African-American and White men. A large-scale prospective cohort with a large proportion of African-Americans that provide questionnaire and blood-specimen data would enable exploration of a wide variety of lifestyle, biochemical, and genetic hypotheses to explain racial differences in prostate cancer and mortality.

The VA provides a unique opportunity to establish a multi-racial cohort with high African-American recruitment (see minority recruitment below). The research and data collection structure in place in the VA system provide an extraordinarily efficient setting for development of an observational cohort study with banked blood samples to evaluate the relationship between lifestyle (including dietary intake), environmental and genetic predictors of prostate disease. Please refer below for detailed discussion of the background on various dietary, biochemical and genetic predictors of prosate cancer that could explain racial variation.

Example 5. Physicians Health Study II

Prostate cancer: Basic and animal research support the possibility that vitamin E may reduce the growth of prostate tumors. Vitamin E (α-tocopherol) is the major lipid-soluble, chain-breaking antioxidant protecting cell membranes from free-radical damage.[8,39-41] *In vitro* and *in vivo* experiments show that the free-radical quenching activity of vitamin E can decrease cancer growth.[15] Vitamin E may also enhance the immune system.[13] Vitamin E has been shown to slow the growth of human prostate tumors in vitro, as well as in rats receiving various doses of chemotherapeutic agents.[23,24,42]

Limited observational data, while not entirely consistent, support the possibility that vitamin E reduces the risk of prostate cancer incidence or mortality. Three case-control studies (conducted in Serbia, Greece, and Uruguay) observed inverse associations between dietary vitamin E and prostate cancer risk,[43-45] with statistically significant reductions of 40% or greater. In the Health Professionals Follow-up Study, conducted among 47,780 initially healthy, middle-aged men, although no overall clear association was found for self-reported vitamin E supplement intake and total, advanced, and fatal prostate cancer, a suggestive reduction in relative risk of metastatic and fatal prostate cancer (RR, 0.44; 95% CI, 0.18-1.07) was observed among ever smokers.[46] One study supports an inverse association which is strongest among those with higher levels of γ-tocopherol.[47] Other cohort[48] and case-control[49-53] studies have not observed significant associations between dietary vitamin E intake or supplemental vitamin E intake and risk of prostate cancer.

The most compelling data suggesting that vitamin E may reduce the risk of prostate cancer come from the Finnish Alpha Tocopherol/Beta Carotene (ATBC) Cancer Prevention Trial. The ATBC was a randomized, double-blind, placebo-controlled trial of α-tocopherol (50 mg daily) and β-carotene (20 mg daily) among 29,133 male smokers. Among those assigned to α-tocopherol supplementation, there was a 32% reduction in prostate cancer incidence ($p < 0.01$) during a median follow-up period of 6.1 years.[54] The effect of vitamin E was apparently strongest on more advanced tumors. A 41% reduction in prostate cancer mortality was also observed. However, since prostate cancer mortality was not a prespecified endpoint, it remains possible that this finding is due to chance.

Definitive proof for the hypothesis that vitamin E reduces the incidence of prostate cancer and risk of death from it will come only from additional large-scale randomized trials. Intriguing results notwithstanding (such as a nonsignificant trend toward reduced risk of prostate cancer among those randomized to vitamin E in the Heart Outcomes Prevention Evaluation trial [personal communication, JL Probstfield, 2001]) other completed trials of vitamin E, including several among individuals at high-risk for cardiovascular disease, were not powered to address the possible benefit of vitamin E on prostate cancer.

One recently initiated study, the Selenium and Vitamin E Cancer Prevention Trial (SELECT), was designed specifically to assess the role of vitamin E and selenium in the prevention of incident prostate cancer among men initially free of prostate cancer based on baseline PSA values and digital rectal exams. PHS II will serve as an important complement to SELECT. PHS II will also contribute unique information regarding vitamin E and prostate cancer. Because the PHS II population is not screened for prostate cancer prior to randomization, the results from this trial should provide information regarding the effect of vitamin E across the spectrum of prostate cancer. If vitamin E

slows the progression of early, undetected prostate cancer, as suggested by data from the ATBC trial and supported by animal studies, there will be greater power to detect this effect in the PHS II than in SELECT. In addition, since baseline blood samples have been provided by 76% of PHS II participants, we have the ability to assess the effect of vitamin E stratified by baseline PSA levels. Thus, we feel that both PHS II and SELECT will provide important and complementary data regarding vitamin E supplementation at doses considerably above recommended daily values and prostate cancer risk. With the proposed extension, PHS II can provide 8.2 years of randomized vitamin E treatment at low cost (approximately $95/randomized participant/year in direct costs).

Table 2. Competed trials of vitamin E and CVD among high-risk individuals using clinical endpoints

Trial	Population	Vitamin E Treatment	Results
CHAOS[131]	2002 men and women with atherosclerosis	400 or 800 IU daily; 1.4 years	Nonfatal MI plus cardiovascular death: RR, 0.53 (95% CI, 0.34-0.83)
ATBC[54]	29,133 male smokers aged 50-69	50 mg daily; 6 years	Nonsignificant 5% reduction in incidence of ischemic heart disease and 16% reduction in ischemic stroke
GISSI[132]	11,324 MI survivors of an acute MI	450 IU daily; 3.5 years	Combined endpoint of death, nonfatal MI, and nonfatal stroke: RR, 0.95 (95% CI, 0.86-1.05)
HOPE[133]	9,541 men and women with existing CVD or at high risk	400 IU daily; 5 years	Combined endpoint of MI, stroke, or cardiovascular death: RR, 1.05 (95% CI, 0.95-1.16)
SPACE[134]	196 hemodialysis patients	800 IU daily; 1.5 years	Composite endpoint of MI, ischemic stroke, PVD, and unstable angina: RR 0.46 (95% CI, 0.27-0.78)

MI = myocardial infarction; PVD = peripheral vascular disease

C. Significance

What about "Significance?" It is your responsibility to state why your work is important. This trickier than it sounds. Things to consider are: (a) What will this study contribute to scientific knowledge? (b) Is there something new and creative with revolutionary potential? (c) Will results lead to knowledge that could reduce morbidity and mortality? and (d) Is the study worth the money? In epidemiology, significance is rarely hard to find, but don't take it for granted. Something more than "cancer is the second leading cause of premature mortality" is not good enough. Increasingly, you must address the cost/benefits of your research. Epidemiology is expensive, especially cohort studies and randomized controlled trials. If you are proposing such a study, you better address up front why it is worth the money. There will be scientists other

than epidemiologists on the review panel, and although money is not supposed to be considered, believe me it is!

Below are excerpts from Significance sections. These illustrate the points above.

D. Significance Examples

Example 1: Cohort Study of Dietary Supplements and Cancer Risk

Overall, chemoprevention and observational studies provide modest evidence for an inverse association between vitamin supplements and cancer risk (4a). The most consistent findings are for vitamin E (7, 62b, 67, 74, 80a), for which the majority of the statistically significant inverse odds ratios were compatible with a 50 percent reduction of risk in users vs. nonusers. Some compounds (e.g., β-carotene) are either ineffective at reducing risk or actually increase cancer incidence and mortality (7, 9, 10).

A large number of Americans are taking supplements (13-16) despite lack of evidence on their benefits or risks, and this provides an opportunity to take advantage of a large "natural experiment." For these reasons, methodologically sound observational studies are needed to investigate the association of supplements with cancer

Between 1973 and 1988 in the United States, there has been a 58 percent increase in the age-adjusted incidence of prostate cancer and an 8 percent increase in mortality.[1] In 1992, prostate cancer was the most common cancer in U.S. men, with 132,000 incident cases and 34,000 deaths.[2] Further increases in prostate cancer incidence and mortality are likely, due to the aging of the US population.[3] Efforts to improve early detection through screening are ongoing, but many prostate tumors diagnosed through screening have already spread beyond the prostate.[4] Primary prevention of prostate cancer is thus a goal of enormous public health importance.

Example 2: Diet and Genetic Risk for Lung and Prostate Cancer

Many prostate cancers are latent, localized lesions found after removal of tissue for treatment of benign prostatic hyperplasia, cystoprostatectomy for invasive bladder cancer, or autopsy. In fact, latent prostate cancer is a very common disease, with estimates of prevalence at autopsy of between 20 and 40 percent.[5,6] It remains unclear whether most incidentally diagnosed, asymptomatic cancers are fundamentally different lesions from aggressive cancers diagnosed symptomatically.[7] *Latent prostate cancers could simply be slow growing tumors that, given sufficient time, will become symptomatic. They could also be tumors that, due to lack of promotional co-factors, will…*

Evidence for Environmental Etiology of Prostate Cancer from Geographic and Migrant Studies

Geographic differences in incidence and mortality suggest an important role of environmental risk factors in the etiology of prostate cancer. There are large (greater than 50-fold) worldwide variations in prostate cancer incidence and mortality, with the highest rates in US Blacks followed by northern Europe and countries with large population….

Dietary Factors Associate with Prostate Cancer

The most consistent evidence for diet and prostate cancer risk is for intake of fat, saturated fat and high fat foods; and intake of fruits, vegetables, dietary fiber, and micronutrients commonly found in high fiber foods. To provide support for including these exposures in the proposed study, we present the evidence linking each exposure to prostate cancer and briefly discuss the proposed biological mechanisms.

Fat, Saturated Fat or High Fat Foods

Attempts to develop animal models for human prostate cancer have met with limited success because of the enormous variation in prostate anatomy, biochemistry, and pathology in lower animals, which seldom develop….

Fruits, Vegetables, Dietary Fiber and Associated Nutrients

Fruits and vegetables contain a host of nutrients (e.g., β-carotene, vitamin C, vitamin E) and other factors (e.g., indoles, protease inhibitors, allium compounds) of a potentially anticarcinogenic nature.[63] Fruits and vegetables are also a major source of dietary fiber, substances of plant origin not metabolized and absorbed in the small intestine. Dietary fiber is also found in cereal products, and is a complex mix of substances, including pectin, hemicellulose and lignin.[57] *Previous research has not carefully separated intakes of fruits, vegetables and dietary fiber, and in the following review we consider evidence for associations between these foods and food components and prostate cancer.*

We point out that the relationship of upper body obesity to serum hormone binding globulin remains strong even after control for body mass index (weight/height squared), suggesting that the association is specific to the pattern of distribution of adipose tissue. Thus, we judge it important to measure obesity and particularly upper body obesity, in order to better understand any associations of dietary fat intake with risk of prostate cancer.

The PCPT assumes that DHT is critical for the initiation and progression of prostate cancer. Finesteride is a 4-azasteroid competitive inhibitor of human 5 α-reductase, and causes a profound reduction in circulating and cellular DHT. Thus, there is a similarity in the causal pathway hypothesized for both the effects of finasteride and a low fat diet: Both a low fat diet and finasteride would reduce the amount of intracellular DHT available for prostate tumor growth. There may be therefore an interaction of diet with the effects of finasteride treatment. Investigators in the PCPT believe that investigating the potential modification by diet of the effects of finasteride is of considerable importance.

Methodologic Issues in Observational Studies of Diet and Prostate Cancer

Previous epidemiological studies have been limited in their ability to find an association of diet with prostate cancer for several reasons.

1. Most previous studies of diet and prostate cancer are retrospective, case control studies. Diet is difficult to measure in case-control studies, because recall of diet in the distant past is subject to considerable bias and inaccuracy.
2. Only one of the cohort studies[56] used a comprehensive measure of dietary intake. The dietary assessment instruments in the remainder of these studies have measured frequency of consumption of as few as two[54] and as many as 35 foods.[55] To measure nutrients such as fat, saturated fat, or percent calories from fat, it is desirable to use a dietary assessment tool that captures the majority of foods usually consumed.
3. The number of cases in previous cohort studies has not been large--ranging from only 63 to 180. Few of these studies have had adequate power to detect the relatively modest relative risks one would expect in observational diet-disease studies.

4. The definition of control or contrast groups for prostate cancer cases is complicated by the high prevalence of latent, undiagnosed disease. Few studies have carefully addressed access to, and use of, medical care by controls, *and no studies have been able to directly and definitively address whether diet is associated with latent tumors, based on uniform biopsy-proven discrimination of persons with and without histological evidence of cancer.*

As will be described in the methods section, this ancillary study to the PCPT offers a unique opportunity to address these limitations.

Summary

In summary, there is considerable evidence that dietary change self-help materials could be effective public health interventions. However, we recognize that quality research in this area is complex. Reasons include: 1) dietary habits appear to be very difficult to modify; 2) sample sizes must be large, due to a combination of small effect sizes and highly variable measures; and 3) research participants are not generally representative of the target population, because only highly motivated people are willing to comply with burdensome, repeated assessments of nutrient intake. We have recently completed a series of studies that address these problems, by developing more potent low-intensity interventions along with assessment tools that measure dietary change with minimal participant burden. Thus, this proposal is unique in two ways. First, we can generate detailed, personalized motivational and behavioral feedback based on self- and telephone-administered questionnaires, avoiding the very high costs of individualized, professionally-administered dietary assessment and counseling. Second, we can evaluate self-help dietary intervention programs delivered to a defined population, using evaluation techniques that are both valid and feasible for public health research.

Example 3: AHRQ Diabetes Proposal

No studies to date have explored biased selection, mortality or FFS costs due to HMO disenrollment in the TEFRA-risk HMO program for Medicare beneficiaries with diabetes. The proposed study will address these issues directly by tracking the five-year experience of a cohort of beneficiaries with diagnosed diabetes enrolled in FFS plans in 1994. By explicitly modeling HMO enrollment and disenrollment, we will be able to provide a clear picture of selection bias and control for potential bias in the causal effect of HMO enrollment on mortality. Using and comparing recently developed panel data methods from biostatistics and econometrics strengthens this proposal in three ways:

1) application of panel data methods that model the repeated measures nature of the HMO enrollment and mortality data;
2) explicit simultaneous estimation of an HMO enrollment equation as part of solving the main (mortality) equation of interest;
3) comparison of the estimated causal effect from a biostatistical method and two econometric methods designed specifically to allow causal inferences in observational panel data.

In addition, by estimating a model of FFS expenditures this study will also examine the effect of HMO disenrollment on costs in the FFS sector. If these high use, high cost beneficiaries can achieve better outcomes (in terms of mortality) in managed care and HMO disenrollment does

not result in higher FFS costs, Medicare may be able to realize significant program savings without a significant reduction in beneficiary health status.

Example 4. VA Biracial Cohort

SIGNIFICANCE TO THE VA

Current estimates are that approximately 176,300 men in the United States will be diagnosed with prostate cancer and 37,000 will die from this disease in 1999. Apart from skin cancer, prostate cancer has become the most common cancer in the United States and is the leading cause of cancer death among elderly US men. Prostate cancer remains a major source of morbidity, mortality, and resource consumption for the VA. The VA is committed to enhancing the health of veterans through prevention and optimal management of prostate cancer and remains dedicated to research in these areas. The prevalence of prostate cancer in this aging male population, the great willingness of patients in the VA Healthcare System to participate in research projects, the existence of accessible national and local administrative and clinical databases, and the existence of an extensive multi-center research infrastructure, provide a unique opportunity to study prostate cancer at a relatively low cost. The establishment of low-cost preventive strategies would be of great benefit to veterans and to the VHA. In addition, better understanding of the causes of prostate cancer could lead to enhanced screening and prognostication as well as improved means of treating and preventing prostate cancer.

E. Preliminary Studies

Overview. The Previous Studies section serves many purposes, and its construction requires considerable judgment and finesse. In the best of all possible worlds, the Previous Studies section will show how your present proposal is a logical next step from your past work. You need simply describe your previous grants, focusing on their methods, results and publications. This is straightforward if you are a well-established, senior investigator in a laboratory science following a relatively linear research path. If, however, you are a new investigator, an established investigator who wants to work in a new area, or an epidemiologist proposing a new study, the Previous Studies section is your chance to convince reviewers that you can do the work **and** that the work as proposed can achieve the specific aims. (Remember your Specific Aims?)

What you choose to include is based on your study. If your specific aims are creative or truly a new idea, then describe the work that leads to the hypotheses. If exposure assessment is critical and difficult, then you should describe work that supports the validity of your instrument. If recruitment is an issue, support how your recruitment method will yield sufficient participants. If the project is large and complicated, describe work that demonstrates your ability to lead such a project. In summary, consider questions reviewers are likely to ask, and then describe your work (and the work of your co-investigators) that addresses those questions.

The best way to address these questions is by presenting data. Data can come from your previously published or ongoing research. Most importantly, data can also come from special

pilot studies completed specifically to support your proposal. Try to complete pilot studies that address both scientific and practical aspects of your proposal. Pilot studies let you estimate response rates and exposure prevalence, validate instruments, estimate effect sizes, and demonstrate your analytic and management competence. The time and money spent on a small pilot or feasibility study is a very good investment!

The best way to present data is in tables, but beware. Tables take a lot of space (but they can be in 10 point type), so make sure the information cannot be more economically put into the text. It is also easy for a reviewer to quickly read and misinterpret a table. If you put data in text it must be read in the context of **your** interpretation.

I can recommend several techniques. First, in a short, introductory paragraph state what your Previous Studies section will cover, and why. This makes it easier for the reader to follow your organization and arguments. Second, make liberal use of headings, subheadings, and sub-subheadings. This too helps the reviewer know the topic being covered, and it saves valuable space compared to using text for transitions between content areas. Third, make it **painfully** clear how your Previous Studies section motivates your specific aims. Do not be shy. Refer back to your specific aims as many times as necessary. Clearly articulated statements leading to a justification of your proposal are very helpful.

Below are examples from Preliminary Studies sections that illustrate the points above.

F. Preliminary Studies Examples

Scientists at the CPRP have extensive experience in the design, implementation and evaluation of both individual- and community-level dietary change studies. To best focus our presentation of this work for this proposal, the description is divided into four sections: a) nutrition intervention research; b) multi-center, diet intervention coordinating center experience; c) self-help dietary intervention trials in primary care practice; and d) methodologic research in intervention assessment.

Nutrition Intervention Research

The Women's Health Trial Feasibility Study (WHT) (CA38551)

Our first, large nutrition intervention study was a clinical trial in which 2064 healthy women were randomized to receive either an intensive intervention to lower fat intake to 20 percent of energy (%en) or no dietary advice. This intervention, consisting of multiple group intervention sessions scheduled over an 18-month period, emphasized nutrition information and behavioral skills necessary to adopt the low-fat eating pattern. Between baseline and six months post-randomization, fat intake in the intervention group decreased from 39.1 %en to 20.9 %en, compared to 38.9 %en to 38.1 %en among controls.[25] Drs. Curry and Kristal were investigators in this study.

Coordinating Center Experience
We are now acting as the statistical and nutrition coordinating center for four, multi-center diet intervention studies. We also provide scientific oversight and consultation to additional studies described below.

The Women's Health Trial: Feasibility Study in Minority Populations (CN15343)

This is a study in which we are modifying the original WHT intervention and assessment protocols for use in a socioeconomically and ethnically diverse group of American women. We have developed and are now validating both our Food Frequency Questionnaire and our Fat- and Fiber-Related Diet Habits Questionnaire in samples of African-American, Latino and lower socioeconomic status women. Dr. Kristal is an investigator in the Coordinating Center and responsible for nutrient intake and diet behavior assessment.

Self-Help Interventions
There are three studies listed below, two with Dr. Shirley Beresford and one with Dr. Susan Curry as Principal Investigator, that strongly relate to the research proposed here.

Randomized Evaluation of Written Personalized Feedback and Financial Incentives as Adjuncts to Self-Help Smoking Cessation Materials (DA04447, Susan Curry, PI)

This randomized controlled trial evaluated written personalized feedback and financial incentive motivational strategies as adjuncts to self-help smoking cessation materials. This was a three-arm study in a sample of over 1,200 smokers recruited from Group Health Cooperative enrollees. Evaluation was at 3 and 12 months, with response rates of 98% and 95% respectively. The written personalized feedback was associated with significantly higher rates of use of the self-help materials and higher cessation rates among program users. Long-term abstinence rates were double those in the comparison groups. The financial incentives did increase use of the materials, but did not result in higher cessation or long-term abstinence rates.

Dietary Intervention Trial in Primary Care Practices (CA 49643)

The North Carolina study served as a pilot for this trial of self-help materials delivered in nine clinics of the Group Health Cooperative of Puget Sound (GHC). In 28 physician practices randomized to intervention or control status, intervention group physicians gave participants a self-help manual titled "Help Yourself," a booklet developed for the study. Evaluation was based on telephone surveys at baseline, three, and twelve months. Dietary assessment included a food frequency questionnaire and the fat- and fiber-related diet habits questionnaire[31] (described in detail in Methods). Overall results are given in Table 1a. At three months, there were statistically significant intervention effects for fat and inconclusive results for fiber; at one year, the intervention effects for both fat and fiber were larger and all reached statistical significance. Intervention effects tended to be larger among women and among participants who were responsible at least in part for food shopping and preparation.

We have completed extensive analyses on the three-month follow-up data related to use of intervention materials (Table 1b). Program effects were strongest among participants who engaged in the active components of the self-help intervention: goal-setting; self-assessment; and recipe use.

Table 1a. Primary Care Practices Diet Intervention Trial
Changes in Fat and Fiber From Baseline to Three and Twelve Months

		Intervention Effect[1]				
		Total	Sex		Food Responsibilities	
			Male	Female	All or Most	Some or None
Fat	3 m	-0.9***	-0.8	-1.0**	-1.1**	-0.7
(% En)	12 m	-1.3***	-0.8	-1.5***	-1.9***	-0.5
Fat[2]	3 m	-0.05***	-0.05*	-0.05***	-0.05***	-0.05***
Score	12 m	-0.05***	-0.04*	-0.05***	-0.05***	-0.04**
Fiber	3 m	0.2	0.1	0.2	0.3	0.2
(g/1000 Kcal)	12 m	0.4*	0.4	0.4*	0.5*	0.2
Fiber[2]	3 m	0.04***	0.04*	0.04**	0.04*	0.03*
Score	12 m	0.04**	0.03	0.04**	0.05**	0.02
(n)		1795	565	1230	1026	768

[1]Intervention minus control, from multiple regression model controlled for baseline value, sex and age.
[2]Scales range from 1 to 3, and are not directly comparable to those on Table 6.
*p<0.05; **p<0.01; ***p<0.001

The booklet also increased the proportion of participants who moved from the precontemplation, contemplation, or decision stages of dietary change at baseline into the action and maintenance stages. For fat, 46% of control versus 62% of intervention participants moved into action and maintenance at three months, and these percentages increased to 53% versus 68% at one year. For fiber, 34% of control versus 45% of intervention participants moved into action and maintenance at three months, and these increased to 38% versus 51% at one year (all control versus intervention differences p<0.01). Overall, these results suggest three areas for further research: 1) How can we increase the use of self-help materials once they are distributed? 2) How can we increase the potency of the materials when they are used? and 3) How can we better design and evaluate interventions to increase the use of fruits and vegetables?

Methodologic Research in Dietary Intervention and Assessment
Given the overall high level of diet-related research activity at the CPRP, we have also completed several methodologic studies directly relevant to methods used in this proposed study.

Determinants of Selecting Diets Low in Fat (CA46695)

This was a methodologic study to develop instruments and methods for community-level dietary intervention research. Products include: 1) a fat-related diet behavior instrument[31]; 2) scales that assess attitudes and beliefs related to selecting low fat diets[32]; 3) a quick dietary assessment

tool for fat and fiber[33]; 4) a Food Use Checklist designed to evaluate community nutrition interventions[34]; and 5) an optically-scanned food frequency questionnaire (FFQ)[35] (Manuscript in press given in Appendix B) with software to generate specific behavioral recommendations.

Table 1b. Primary Care Practices Diet Intervention Trial
Changes in Fat and Fiber From Baseline to Three Months, by Use of Intervention Materials

	Fat (% En)	Fat Score	Fiber (g/1000 Kcal)	Fiber Score	N
Control Group Only					
Total	-0.5	-0.03***	0.3**	0.03***	991
Intervention Group Only					
Total	-1.4***	-0.08***	0.5***	0.07***	896
Read Intervention Materials					
Yes	-1.6	-0.09[b]	0.6	0.07[b]	798
No	-0.4	-0.032	0.3	0.00	97
• **If materials were read:**					
Found Useful					
Very	-2.6[b]	-0.14[b]	1.0	0.11[a]	224
Somewhat	-1.5[a]	-0.09[b]	0.4	0.06	464
Not at all	-0.4	-0.01	0.5	0.04	110
Used Recipes					
Yes	-2.9[b]	-0.14[b]	1.2[b]	0.09	120
No	-1.4	-0.09	0.5	0.07	678
Completed Written Section					
Yes	-2.1	-0.14[b]	0.8	0.12[b]	149
No	-1.5	-0.09	0.5	0.06	649
• **If written section completed:**					
Completed Self-Evaluation					
Yes	-2.4	-0.14	1.1[a]	0.12	120
No	-0.5	-0.12	-0.2	0.12	29
Completed Goals					
Yes	-2.9[a]	-0.16	1.5[b]	0.14	83
No	-1.0	-0.10	0.0	0.10	66

[1]From Multiple Regression Model, controlled for baseline value, gender and age.
[2]Scales range from 1 to 3, and are not directly comparable to those on Table 6.
*p<0.05; **p<0.01; ***p<0.001
[a] Contrast vs. no/not at all p<0.05; [b]Contrast vs. no/not at all p<0.01

Promoting Dietary Change in Communities: Applying Existing Models of Dietary Change to Population-Based Interventions (CA 53712)

Finally, the CPRP sponsored a conference that addressed the development and implementation of population-based interventions and strategies for dietary change. Proceedings of this conference have been broadly disseminated.[38]

Methods to Assess Dietary Change

Our methodologic research related to assessing dietary change requires further elaboration. We believe that the assessment of dietary change, especially in studies of public-health interventions, is best achieved by measuring the specific behavioral targets of the intervention. While in clinical research it may be feasible to measure nutrient intake by collecting multiple day food records or multiple FFQ's, these assessments carry a large participant burden. We have attempted to use FFQ's in public health intervention studies, but observed response rates as low as 30 percent. The integrity of the results based on these survey data is compromised, because survey respondents are likely a very biased sample of persons exposed to the interventions.

Our first attempt to extend the FFB to include fiber-related habits was in the Dietary Intervention Trial in Primary Care Practices, where we added items on use of fruits, vegetables and whole-grain products. In this study, the correlation at baseline of the FFB fat scale with the FFQ %en fat was 0.55; the correlation was 0.49 for fiber (g/1000Kcal) (both n=2116, $p<0.001$). As part of this study, we also completed a validation substudy of 110 participants who completed four-day food records as well as the telephone-administered FFQ and FFB at baseline and one-year. Baseline data (Table 2) confirm our previously published results on the validity of the FFB to assess fat.[27,31] For fiber, the correlations between the FFB fiber score and nutrient intake measures were equally strong. A manuscript in press[47] (Appendix C) gives more detailed results of this substudy.

Table 2. Validity of Fat-and Fiber-Related Scores in the Primary Care Practices Intervention Trial.

Correlations at Baseline Among 4-Day Record, Food Frequency Questionnaire (FFQ) and Fat- and Fiber-Related Habits Scale Scores. (N=110)

	Fat (% EN)			Fiber (g/1000 kcal)	
	4-Day Record	FFQ		4-Day Record	FFQ
Fat Scale	.47	.60	Fiber Scale	.56	.58
4-Day Record	--	.58	4-Day Record	--	.58

More Previous Studies Examples

Te scientists contributing to this study have extensive experience in the design, conduct and analysis of epidemiologic studies of cancer of the breast, colon, lung, prostate, kidney, bladder, uterus, and melanoma, and in the measurement of a range of exposures (see Biosketches). While much of this experience will contribute to this proposed study, we outline below only our

most relevant experience in the conduct of studies of the relation of supplement intake to cancer incidence, in the conduct of large scale studies (cohort and clinical trials), and in the measurement of supplement use and dietary intake. We also give the results of our pilot study on recruitment.

Studies of supplement use and cancer. Dr. White has conducted (as PI or chair of student dissertation research) three case-control studies on supplement use and cancer risk, one on colon cancer (82), one on bladder cancer (83), and one on melanoma (84). All three assessed use of multivitamins (by type), and use of individual supplements of vitamin A, C, E and calcium during a 10 year reference period. The exposure measures were similar to those proposed here: Average daily dose of supplements over the 10 year period was calculated as a function of years of use in the 10 year period, days per week of use in those years, and usual brand or type (for multivitamins) or reported units per day for individual supplements. All three studies found use of multivitamin supplements to be associated with reduced risk of cancer, but only among those who reported long term use. *For colon cancer, the risk ratio (RR) was 0.49 (95% CI 0.35-0.69) for 10 years of daily use vs. none (82); for bladder cancer, RR=0.39 (0.24-0.63) for 10 years daily use (83); and for melanoma, RR=0.69 (0.43-1.1) for the equivalent of 5 years daily use over the 10 year period (84). The strongest of the associations observed were a reduced risk of colon cancer associated with supplemental vitamin E intake among women (RR=0.43 (0.26-0.71) for use of 200+ IU on average/day over the 10 years), and a reduced risk of bladder cancer associated with supplemental vitamin C (RR=0.40 (0.21-0.76) for use greater than 500 mg/day). For each of these, a dose-response gradient was observed (p for trend all <0.01), and for each, the highest category of use represented intake that can be achieved only by use of individual supplements.* These studies have given us experience in the issues involved in the design and analysis of studies of supplement use, and provide support that detailed assessment of long-term supplement use might be important. However, these studies were limited by the problems of case-control studies, particularly the issue of selection bias noted above.

Dr. Potter was one of the Co-Investigators of the Iowa Women's Health Study, a cohort study of 35,000 women, for over eight years while at the University of Minnesota. He was involved in all aspects of the study, including the analysis of the dietary and supplement data. He was one of the co-authors on the study of the role of α-tocopherol, calcium, and vitamin D supplements in colon cancer from that study (67), as well as being the Principal Investigator of the Minnesota randomized trial of calcium and rectal epithelial proliferation (45), both described in Background, above. In addition, Dr. Potter is co-chair of a workshop to be held in January, 1997 on the Evaluation of Dietary/Nutritional Exposure and Genetic Susceptibility in Studies of Cancer Etiology.

One of the major randomized clinical trails of supplement use, The Carotene and Retinol Efficacy Trial (CARET), was designed and coordinated at the Fred Hutchinson Cancer Research Center (FHCRC). Results of this study (10) were reported in Background. Our involvement is described below.

Exposure measurement. Dr. White is co-author of the book Principles of Exposure Measurement in Epidemiology, for which she was the primary contributor to the sections on the design and analysis of validity and reliability studies and on quality control procedures (81). She has also

shown that one approach to reducing the effects of measurement error is to recruit a study population with a large exposure variance (85), which is relevant to the design of the proposed study.

Dietary Assessment**.** Drs. Kristal and Patterson have been responsible for dietary assessment in many studies of diet and cancer, four of which are very large trials (>15,000 participants each). Dr. Kristal was responsible for the design and early evaluation of dietary assessment methods in CARET. Drs. Patterson and Kristal were investigators in the Coordinating Center for The Working Well Study, a randomized trial of worksite-based health promotion intervention of over 25,000 workers in 124 worksites located throughout the United States. In this study we developed a new food frequency questionnaire (FFQ) and were responsible for dietary assessment activities. Drs. Kristal and Patterson are Co-Principal Investigators of A Prospective Cohort Study of Diet and Prostate Cancer, a diet ancillary study to the Prostate Cancer Prevention Trial (see study description below). We developed a FFQ appropriate for this study population and are completing a validity study on this form. Drs. Patterson and Kristal provide scientific oversight of dietary assessment activities in The Women's Health Initiative (WHI). The WHI Clinical Trial and Observational Study are components of a nine-year prospective research program designed to address the issues of diet, hormone replacement, and chronic disease in 157,000 mature women. Dr. Patterson took the lead in the design of the WHI FFQ and is the principal investigator of a study of its validity. She is also responsible for a WHI study of measurement characteristics of the FFQ across racial/ethnic groups.

*Dietary Supplements**. Dr. Patterson has recently published or submitted four manuscripts on the measurement of vitamin supplement use or on correlates of use (80b, 80c, 85a, 85b). One of these studies was an NIH funded study on Methods for Practical and Accurate Assessment of Vitamin Supplements. This validation study compared supplement data collected in a telephone interview and from a self-administered questionnaire with data derived from a detailed in-person interview and transcription of the labels of supplement bottles (i.e., a gold standard) among adult supplement users (n=104) (85a). Spearman correlation coefficients comparing average daily supplemental vitamin and mineral intake from the interview or questionnaire to the gold standard ranged from 0.76 (95% CI 0.66-0.83) for vitamin C to 0.08 (95% CI -0.14 to 0.29) for iron, with a mean of about 0.5. The principal sources of error were inaccurate assumptions regarding the micronutrient composition of multivitamins and respondent confusion regarding the distinction between multiple-vitamins and single supplements. These results indicate that carefully designed assessment forms and supplemental nutrient databases are needed to accurately assess supplemental nutrient intake. We used information from this study to inform the design of the supplement questionnaire proposed for this study. In another study, we used data from a random-digit-dial survey on cancer risk behavior in adults (n=1,449) to identify cancer-related behaviors associated with supplement use (80b). We found that among women, supplement users were more likely to have had a sigmoidoscopy, hemoccult test, or mammogram in the past two years. Among men, supplement users were twice as likely to have had a PSA test and to regularly take aspirin. Supplement users were statistically significantly more likely to exercise regularly, eat four or more servings fruits and vegetables per day, follow a low-fat diet pattern, and believe in a connection between diet and cancer. This investigation will help ensure that we collect data on potential confounders of the associations between supplement use and cancer risk. We also used data from our pilot study (described below) to investigate whether a*

one-time measure (i.e., current use) of supplement use is a valid proxy for long-term (10 year) supplement use (80c). We found that current supplement use gave an upwardly biased estimate of long-term micronutrient intake from supplements and that, among supplement users, current use explained only 50 percent of the variability in micronutrient intake from supplements over the past 10 years. We concluded that this measurement error may explain many of the null associations seen in our review of vitamin supplements and risk of cancer (4a).

Measurement of serum nutrients. Dr. King, a nutritional biochemist, develops and supervises various nutrient analyses performed in the Core Laboratory of the FHCRC. The primarily function of the Core Laboratory is to provide expert advice and laboratory analyses for large-scale epidemiologic studies. During the last 3 years over 11,000 serum specimens were analyzed for retinoids, carotenoids and tocopherol for the CARET study alone. Also for the CARET study, Dr. King expanded the original carotenoid assay to include lycopene, lutein, xeaxanthin, cryptoxanthin, and γ-tocopherol. In the past, she implemented fluorometric assays for thiamine and riboflavin and she measured trace elements in animal diets and tissues using an inductively-coupled argon plasma spectrophotometer (ICP) methods (see Biosketch). Dr. King is also assisting with the establishment of trace element analyses for selenium, zinc and magnesium at the University of Zimbabwe as part of an NIH-funded international study.

Collection of buccal cells and DNA extraction. Drs. Potter and King have experience in the collection of buccal cells for DNA and nutrient analyses in both a large trial (86) and in smaller metabolic studies (87). Dr. Potter was the epidemiologist on the investigative team that originally proposed and developed the collection of buccal cells for studies of human metabolism (88). As Co-Investigator of the Minnesota Breast Cancer Family Study (86), Dr. Potter was involved in the collection of buccal brushings for DNA, which have proven to be an excellent source of high molecular weight DNA. Richards and others (89,90) have shown that the DNA obtained from buccal cells for PCR reactions need not be pure and does not require lengthy classic DNA extraction methods to result in a 99% success rate for the CFRT genotype that they assessed. Dr. Potter and his colleagues currently use the Richards procedure to obtain DNA for the NAT2 genotype. *Dr. King recently tested the proposed methods for buccal cell collection and DNA extraction for this study (see below).*

Supplement Pilot Study. In preparation for this proposal, we conducted a pilot study to test the proposed recruitment plan, including our ability to recruit a cohort with a large percentage of supplement users, who were willing to complete lengthy questionnaires and to join a study in which they would be followed through health registries (as was explained in the recruitment letter). The cover letter and two questionnaires (Appendix A and B) were mailed to 800 randomly selected age- and county-eligible individuals from a mailing list obtained from a commercial firm, Research and Response. Our response rates were 45% (186 of 414) for women and 38%(147 of 386) for men, including non-response due to undeliverable mail. We tested two versions of the recruitment letter, one that specifically targeted supplement users, and a second more general letter inviting recipients into a study of diet, supplements, and health. Both letters were successful in recruiting a sample with 75% or more supplement users, and our proposed cover letter will include elements of both letters. We also attempted to recruit participants through " health food" stores, which yielded a poor response rate. The results presented in Table 5 are on all respondents to the mailed recruitment. More than half of the female respondents took a multivitamin pill with minerals, 75% used supplemental vitamin C (from individual or multivitamin pills), 71% vitamin E, and 76% calcium. Use among men was lower, ranging from 31% for multivitamin pills plus minerals to 55% for vitamin C. On average, respondents who took supplements used them for 6 of the past 10 years. Because we plan to analyze our results across four categories of use (none, and tertiles of use among users), we present the 33rd and 67th percentile of daily dose over the 10 year period among users. Those in the upper third of vitamin C use had intakes at least 5 times the RDA of 60 mg and those in the upper third of vitamin E had intakes at least 4 times the RDA of 30 mg.

Table 5. Supplement Use of Respondents to a Pilot Study in Western Washington State

Use in past 10 years	Females	Males
Any type supplement (%)	87.7	66.1
Any type multivitamin (%)	66.7	46.8
Multivitamins with minerals		
Users (%)	52.6	30.7
Years taken among users (mean ± sd)	6.0 (3.6)	5.7 (3.7)
Vitamin C		
Use as single supplement (%)	48.5	29.9
Years taken as single supplement (mean ± sd)	6.8 (3.4)	7.3 (3.1)
Total use[a] (%)	74.9	55.1
Tertiles of dose over 10 years[c] (mg/day)	70/417	50/337
Vitamin E		
Use as single supplement (%)	36.5	25.2
Years taken as single supplement (mean ± sd)	6.0 (3.5)	5.5 (3.8)
Total use[a] (%)	71.3	52.0
Tertiles of dose over 10 years[c] (IU/day)	26/170	23/130
Calcium		
Use as single supplement (%)	59.6	15.7
Years taken as single supplement (mean ± sd)	5.9 (3.5)	6.3 (3.5)
Total use[b] (%)	75.4	39.4
Tertiles of dose over 10 years[c] (mg/day)	142/427	49/141

[a]From multivitamins, multivitamins with minerals, stress supplements, antioxidant mixtures, and single supplements.
[b]From multivitamins with minerals, single supplements (including Tums and OsCal), and calcium/magnesium/zinc mixtures.
[C]33rd and 67th percentile among users

Pilot Studies of Collection and Processing of DNA from Buccal Brushings. *We conducted pilot studies to test if we could obtain buccal cell DNA using cytology brushes sent to participants in the mail. Using names of respondents from the Supplement Pilot Study, a mailing was sent to randomly selected men (n=20) and women (n=20) age 50-74 in the 13 counties of western Washington State. The mailing included a cover letter, consent form, instructions, and a packet of three cytology bushes (labeled and wrapped in bubble wrap). Participants were instructed to rinse their mouths with tap water and scrape their right cheek with brush 1, left cheek with brush 2, and use brush 3 for scraping either one or both cheeks. The instructions also specified to twirl*

the brush while rotating the brush downward. Finally, participants were asked to collect brushes within 1 week of receiving the mailing and to return the brushes packaged in bubble wrap in the prepaid return envelope via ordinary mail. Upon receipt, brushes were stored immediately in a -70C freezer.

After the initial mailing and one reminder, we received samples from 11 women and 10 men for a 53% response rate. We also collected 10 specimens using similar procedures on local (Hutchinson Center) volunteers.

As a preliminary step, we tested two DNA extraction kits: QIAamp blood kit (Qiagen Co., Valencia, CA) and MasterAmp buccal swab DNA extraction kit (Epicentre Technologies Co., Madison, WI). We found the QIAamp procedure to be much more successful. After one week of -70℃ freezer storage, brush number 1 from each participant was used for DNA extraction and DNA verification on gel electrophoresis on both sets of subjects. Gel electrophoresis rather than OD_{260} was used to determine the approximate amount of DNA because this method requires less DNA, and it shows whether the DNA is of good quality. DNA was obtained from 17 of 21 brushes (81%) from the Supplement Study Pilot participants and all 10 of the local participants, with a DNA yield range of approximately 0.5 to 1 μg. Assuming that 10-100 nanograms are needed for PCR genotyping, this should be sufficient DNA for 5-50 tests.

PCR amplification (for epoxide hydrolase requiring short DNA pieces (0.3kb) and NAT2 requiring longer DNA fragments (1.1kb) was tested on the 10 local samples only. Two different sizes were chosen because amplification of longer fragments requires better quality DNA than amplification of short fragments. The epoxide hydrolase amplification was successful for all 10 (100%), and the NAT2 was successful on 7 (70%). The PCR was based on $1/10^{th}$ of the DNA from one brush, so repetition of the test should lead to higher success rates. By modest enhancements to each step of these procedures, we expect that we could get the return rate of the brushes to 55%, DNA yield to 85% and PCR success for those with some DNA to 90% on average, for an overall response rate of 42% (conservative estimate).

Management of Large Cohorts. The CARET study demonstrates our expertise with managing large, complex studies and their associated databases. Dr. Thornquist is the primary statistician for the trial and the Director of the CARET Coordinating Center. During the intervention phase of the trial, the 18,000 participants were contacted 3-4 times per year for extensive data collection, including trial endpoints, lung cancer risk factors, and dietary intake. The current size of the CARET database is 525 Mbytes. As of the last analysis, over 98% of participants were still in active contact with CARET staff.

Dr. White is the Scientific Liaison for the Women's Health Initiative Observational Study (OS). In that capacity, she works with the national OS advisory committee in developing the forms and procedures for the study and coordinates the implementation of these through the WHI Coordinating Center at the FHCRC. This involves development of questionnaires on risk factors for a wide range of disease outcomes. It also involved the development and oversight of implementation of the annual mailings to the 100,000 cohort women that are conducted in-house at the WHI Coordinating Center. Through multiple mailings to each women, we have achieved

an 85% response rate, after which the 40 clinical centers conduct telephone interviews of non-respondents.

Drs. Kristal and Patterson are Co-Principal Investigators of a diet ancillary study to The Prostate Cancer Prevention Trial (PCPT) . The PCPT is a Phase III, randomized, double-blind, placebo controlled trial in 18,000 men, aged 55 to 70, of the drug finasteride (Proscar®) for the primary prevention of carcinoma of the prostate. The diet study will investigate whether dietary fat, fruits and vegetables, or micro-nutrients are associated with the risk of prostate cancer. This study uses the DataFax system, an image-based data entry and editing system, similar to the one proposed to be used here, to accurately and efficiently process the large volume of study forms. The investigators re-designed the food frequency questionnaire for this system. They also wrote the procedures for quality control of dietary and anthropometric data collection occurring at over 250 clinical sites.

Experience with SEER. As noted above, Dr. Potter has many years of experience as Co-Investigator of The Iowa Women's Health Study. This study demonstrated that linkage of a cohort recruited from a defined area to a SEER registry covering that area, is an accurate and cost-effective approach to follow a cohort for cancer endpoints. Dr. White is co-PI of a project that links information on risk factors, medication use, mammography and breast pathology reports with the SEER registry among 92,000 women over age 35 who are members of an HMO. She has published numerous studies based on the SEER data (see Dr. White's Biosketch). This experience with the use of SEER registries will allow us to effectively link to and interpret the cancer incidence information.

In summary, we have experience in studies of all of the major cancers (lung, prostate, breast and colon), have developed tools for assessment of diet and supplement use, and have demonstrated expertise in the technical complexities of conducting large-scale studies, including cohort studies. In addition, our Pilot Study supports that the methods we propose for recruitment of a cohort of supplement users will be successful.

Alternative Therapies for Back Pain

The study team has extensive experience with evaluating interventions for low back pain (Cherkin, Deyo). Dr. Cherkin (Principal Investigator for the proposed study) was the co-Principal Investigator on the Low Back Pain Patient Outcome Research Team (PORT), a major 5-year program project that has resulted in approximately 100 publications. Richard Deyo, MD, MPH, (Co-investigator on proposed study) was the Principal Investigator for the Low Back Pain PORT. Drs. Cherkin and Deyo and Project Director Janet Street, MN, CPNP, have completed one randomized trial comparing two educational interventions with usual care alone (Cherkin, 1996) and are currently involved with two other trials. One trial compares chiropractic and physical therapy and the other compares different methods of providing information to help patients decide whether or not to have lumbar surgery. The outcome measures and follow-up strategies that have been successfully used in these studies are similar to those proposed for use in this study. These trials have all been conducted at Group Health Cooperative of Puget Sound which continues to be an excellent and enthusiastic laboratory for these types of studies.

The study team's expertise in the area of low back pain is complemented by the expertise of Drs. Eisenberg and Kaptchuk in the areas of alternative medicine in general and with acupuncture in particular. Dr. Eisenberg is a general internist who has gained national attention as a leading expert on alternative medicine since the publication of his article in the New England Journal of Medicine three years ago documenting the widespread use of unconventional medicine in the United States (Eisenberg, 1993a). Dr. Eisenberg is currently director of the Harvard Center for Alternative Medicine Research and has been an advisor to the NIH Office of Alternative Medicine since it was established in 1992. Dr. Eisenberg's research has focused on the effects of alternative treatments such as relaxation and cognitive behavioral techniques on outcomes of care for hypertension (Eisenberg, 1991, 1993b) and has written a book on Chinese medicine (Eisenberg, 1985). Dr. Kaptchuk is an acupuncturist with a doctorate in Chinese Medicine. He has written one book on Chinese medicine and another on healing (Kaptchuk, 1983, 1986). Dr. Kaptchuk is an Instructor in Medicine at the Harvard Medical School and Associate Director of the Center for Alternative Medicine Research.

Drs. Eisenberg, Kaptchuk and Cherkin are currently planning a randomized trial to determine if patients with acute back pain who can choose from among usual medical care, acupuncture, massage and chiropractic have better outcomes (symptoms, dysfunction, disability, satisfaction and costs) than those who have no choice beyond usual medical care. This "preference" trial is not designed to compare the relative effectiveness of the alternative therapies. In preparation for this trial, Drs. Eisenberg and Kaptchuk have been working closely with the American Massage Therapy Association and the American Association of Acupuncture and Oriental Medicine to develop treatment protocols that clearly describe the forms of treatment that will and will not be used in their study. These protocols, which will be finished by September, 1996, will be made available to Dr. Cherkin for use in the proposed study. Few, if any, modifications will be made in the nationally-developed protocols to accommodate local requirements.

Dietary Assessment using a Questionnaire

A self-administered food frequency questionnaire (FFQ) (**Appendix A**) was mailed to all PHS II participants in two phases. First, physicians from PHS I who continued on to PHS II were sent FFQs in April 2000. Second, new physicians recruited for PHS II were sent FFQs during the run-in phase of the trial prior to randomization. Overall, of the 14,642 randomized men, 13,324 (91%) have completed and returned the FFQ. This questionnaire, developed by Walter Willett, MD, DrPH, and colleagues, is an efficient, reliable, and accurate instrument for categorizing individuals according to their intake of 32 nutrients, including vitamin E, vitamin C, and folic acid.[249-251] Data from the FFQ will be used to categorize the participants according to their baseline intake of various nutrients and then to evaluate whether the effect of each randomized treatment varies according to baseline dietary intake